

REVIEW ARTICLE

# Cell growth and differentiation in *Arabidopsis* epidermal cells

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## Abstract

Plant epidermal cells are morphologically diverse, differing in size, shape, and function. Their unique morphologies reflect the integral function each cell performs in the organ to which it belongs. Cell morphogenesis involves multiple cellular processes acting in concert to create specialized shapes. The *Arabidopsis* epidermis contains numerous cell types greatly differing in shape, size, and function. Work on three types of epidermal cells, namely trichomes, root hairs, and pavement cells, has made significant progress towards understanding how plant cells reach their final morphology. These three cell types have highly distinct morphologies and each has become a model cell for the study of morphological processes. A growing body of knowledge is creating a picture of how endoreduplication, cytoskeletal dynamics, vesicle transport, and small GTPase signalling, work in concert to create specialized shapes. Similar mechanisms that determine cell shape and polarity are shared between these cell types, while certain mechanisms remain specific to each.

**Key words:** Actin, *Arabidopsis*, auxin, endoreduplication, GTPase signalling, microtubules, morphogenesis, morphology, pavement cells, planar polarity, root hairs, trichomes, vesicle trafficking.

## Epidermal cells: growth and function

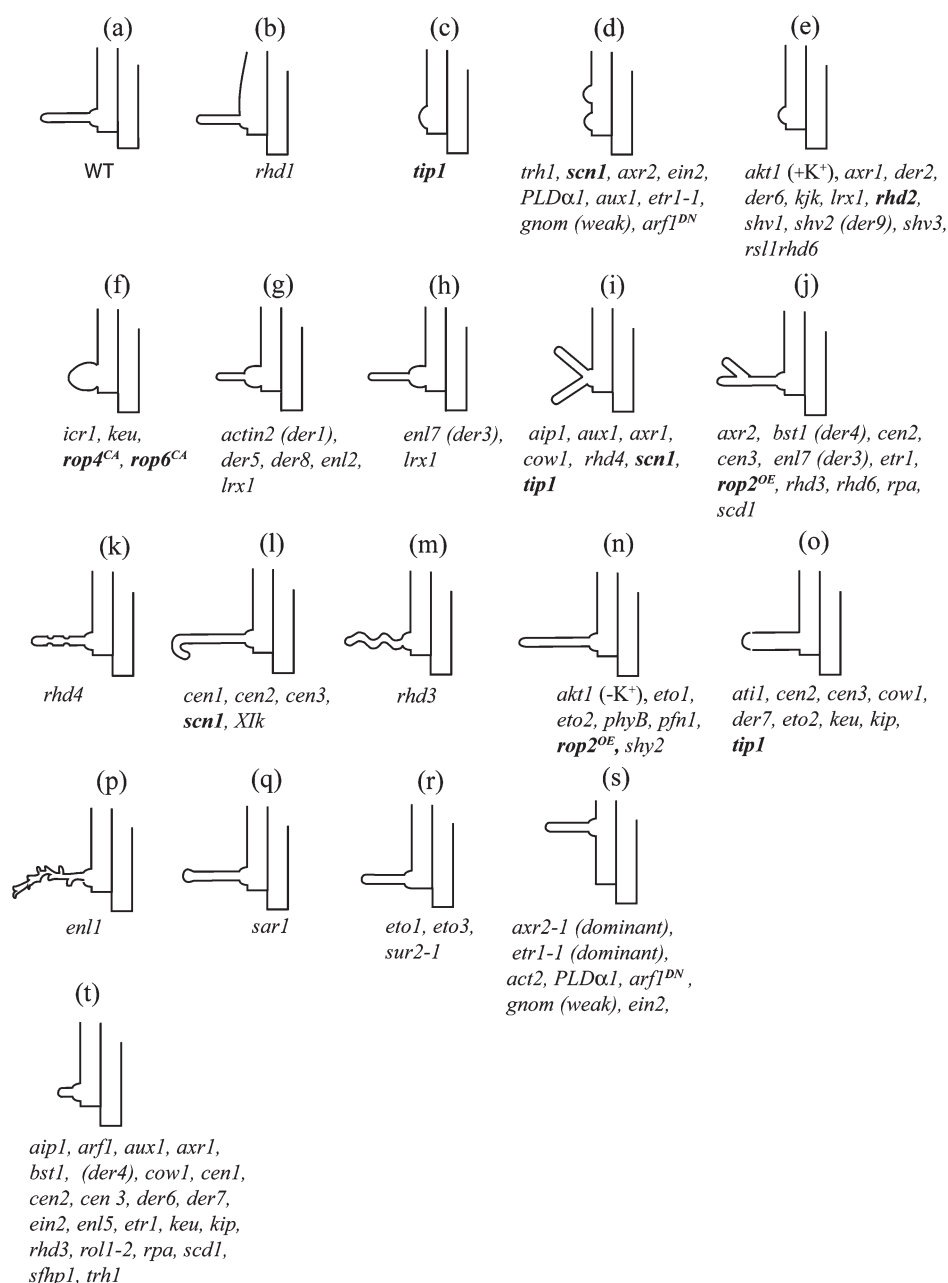
**Teaser:** *Arabidopsis* epidermal cells are a paradigm system for the study of fundamental genetic, molecular, and cell biological processes that are involved in cell morphogenesis.

The highly specialized cell morphologies of *Arabidopsis* trichomes, root hairs, and pavement cells make important

contributions to the overall function of the epidermis. Unicellular trichomes protrude from specific positions on the leaf surface and their shape is largely invariant, forming three or four branches that emerge after central stalk development (Fig. 2). In comparison to surrounding epidermal cells, trichomes are enormous, often exceeding 1 mm in length, and help to function as ‘barbed wire’ against herbivore attack (Melaragno *et al.*, 1993). Despite their highly polarized structure (pointed branches), trichomes are considered to grow by diffuse growth and not tip growth (Schwab *et al.*, 2003). Their tremendous size is correlated with their ploidy level, as mature trichomes normally contain a DNA content between 32C and 64C, obtained via successive endoreduplication rounds (Sugimoto-Shirasu and Roberts, 2003). Root hairs, another highly specialized cell type, function to increase the surface area in contact with the rhizosphere, acquire water and nutrients, provide anchorage, and mediate various plant–microbe interactions (Grierson and Schiefelbein, 2002). Like trichomes, they also greatly increase in size during development and some evidence suggests that in *Arabidopsis* they may also endoreduplicate (Sugimoto-Shirasu *et al.*, 2005). Unlike trichomes, root hairs do not form branches but rather exhibit unidirectional polarized growth (Fig. 1). Pavement cells, the most abundant epidermal cell type, are morphologically unspecialized and primarily function to protect layers beneath the epidermis (Ramsay and Glover, 2005). These irregularly shaped cells contain protruding lobes that interdigitate with their neighbouring pavement cells, together forming a complex, interlocked, puzzle-like pattern. Endopolyploidy is common but not universal in pavement cells, with ploidy levels ranging between 2C and 16C (Melaragno *et al.*, 1993).

As a cell develops, it co-ordinates expansion with polarized growth to determine overall cell shape. During cell growth, turgor pressure is created by an increase in

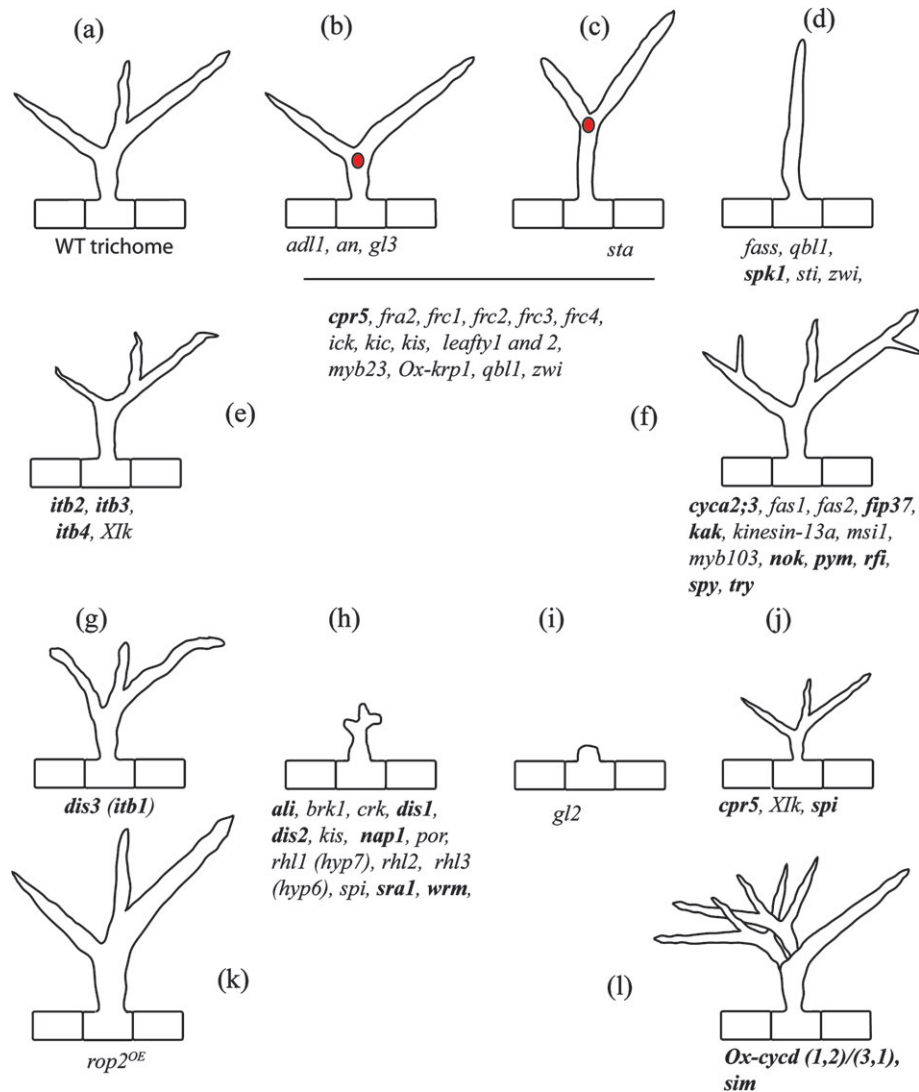
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**Fig. 1.** Phenotypes of root hair morphogenesis mutants. The phenotypes are illustrated with the corresponding gene mutant abbreviations. Mutants in bold face have been described in the text. (a) Wild-type root hair; (b, c, f, g, h) mutants displaying varying alterations in the development of bulge formation; (c, d, e) mutants with an inhibition of tip growth; (g, t) mutants with a reduction in tip growth; (h, n) mutants with an enhancement of tip growth; (d, i, j, p) mutants displaying a loss of polarized tip growth; (n, o) mutants with altered hair width; (r, s) mutants with altered planar polarity. The hair cells have not been drawn to scale. This figure has been modified from Grierson and Schiefelbein (2002), copyrighted by the American Society of Plant Biologists and reprinted with permission.

vacuolar volume, which causes the cell to expand isotropically. Distinct morphologies are reached via anisotropic expansion that is achieved by localized cell wall extension and targeted secretion of plasma membrane and wall components. Thereby, the internal turgor pressure is allowed to push upon the weakened wall creating a site of polarized growth. The bulge that subsequently forms then becomes a discrete site where the growth machinery is

presumably targeted. Therefore, cell morphogenesis can be viewed as a highly regulated interplay between turgor-mediated cell expansion and cell wall-mediated control of internal pressure to create polarized growth. Conventionally, growth is separated into two categories: diffuse growth and tip growth. Diffuse-growing plant cells display even expansion over a large surface area, while tip-growing cells have a restricted site of expansion resulting in



**Fig. 2.** Phenotypes of trichome morphogenesis mutants. The phenotypes are illustrated with the corresponding gene mutant abbreviations. Mutants in bold face have been described in the text. (a) Wild-type trichome; (b) mutants affected in secondary branch initiation; (c) mutants affected in primary branch initiation. In (b, c) the red dot represents the position of the nucleus. Mutants listed under the black line have not been characterized for their role in primary or secondary branching. (d) Branchless mutants; (e) mutants with altered branch length and position; (f) supernumerary branch formation; (g–i) ‘distorted’ or arrested trichome development; (j, k) overall trichome size is affected; (l) multicellular trichome. The cells have not been drawn to scale. The figure has been modified from Schellmann and Hulskamp (2005) and is reproduced by kind permission of UBC Press.

extreme polarization (Mathur, 2004). Cells can display different degrees of growth polarity that vary between diffuse growth and tip growth. New research reveals that this dichotomous view of cell growth may not address important aspects in overall cell shape determination because similar molecular mechanisms might be involved in both types of growth. In *Arabidopsis* epidermal cells, it is becoming apparent that both diffuse growth and tip-growth may be orchestrated by components of the same cellular machinery (Mathur, 2006).

In recent years, much attention has been given to the role of various cellular processes in the establishment of cell shape: endoreduplication, cytoskeletal dynamics, vesicle transport, and small GTPase signalling. This

review aims to present an overview of the genetic, molecular, and cell biological processes involved in epidermal cell shape formation, highlighting the 90+ loci that have been identified in *Arabidopsis* contributing to epidermal cell morphogenesis. As the individual roles of all the genes implicated cannot be discussed in the scope of this review, a table listing the loci involved has been provided (see Table S1 in the Supplementary data at *JXB* online). This table also gives complete gene names and descriptions as gene names often appear only as abbreviations in the text of this review. In addition, Figs 1 and 2 illustrate the corresponding mutant phenotypes for all genes found in Table S1, primarily for non-specialist readers who are new to research utilizing these model cells.

## Role of endoreduplication in cell size control and trichome branching

One facet of cell morphology that remains largely unsolved is how cells regulate their size. Yeast and animals cells can double their size while plants can increase cell size by >1000-fold during post-mitotic development (Sugimoto-Shirasu *et al.*, 2005). Plants can achieve such tremendous cell size by increasing ploidy levels via endoreduplication. This process occurs when the nuclear genome is duplicated without accompanying mitotic cytoplasmic division and often correlates with an increase in both nuclear volume and cell size (Sugimoto-Shirasu and Roberts, 2003). In order to keep up with the metabolic demand of large cells, it is theorized that plants utilize this mechanism to provide enough genetic material for increased protein synthesis requirements (Sugimoto-Shirasu and Roberts, 2003). This suggests that the endomitotic cycle arose as an alternative to the mitotic cycle, providing a selective advantage for plants undergoing rapid growth (Melaragno *et al.*, 1993). How final cell size is determined in plants remains largely unclear but studies utilizing trichome cells as a model provide some clues towards elucidating how endoreduplication might be utilized to achieve such colossal growth.

Trichome branch number often correlates with the number of rounds of endoreduplication the cell has undergone. Mature wild-type trichomes normally form three branches but there can be some natural variation (up to four branches) within the different *Arabidopsis* ecotypes (Passardi *et al.*, 2007). Several branching mutants have been obtained whose branch number can range from branchless up to six branches or more (Perazza *et al.*, 1999) (Fig. 2). Generally, those with fewer branches (none to two) display lower ploidy levels and those with more branches (five plus) display higher ploidy levels, although there are also mutants with altered branch number and normal DNA content. The correlation between the numbers of branches formed and the number of endocycles completed suggests that endoreduplication plays a role in branch initiation. Confirming this idea, numerous genes involved in endoreduplication have been identified among these trichome mutants. One group of genes has been shown to act as negative regulators of branching events: *kak*, *nok*, *pym*, *rfl*, *spy*, and *try* mutants exhibit an increase in branch number (Fig. 2) (Hulskamp *et al.*, 1994; Folkers *et al.*, 1997; Perazza *et al.*, 1999; El Refy *et al.*, 2003). For *kak*, *pym*, *rfl*, and *try*, the supernumerary branches are due to an increase in endoreduplication (Hulskamp *et al.*, 1994; Perazza *et al.*, 1999). KAK was shown to function as a HECT domain-containing putative E3 ligase that possibly ubiquitinates proteins that normally promote the progression of endocycles (El Refy *et al.*, 2003). However, the function of PYM, RFL, and TRY in promoting endoreduplication remains unknown.

Furthermore, no studies address whether these loci affect endoreduplication in pavement cells.

Trichome mutant screens identified additional genes influencing cell cycle control. *Arabidopsis* trichomes are normally unicellular; however, the recessive *sim* mutant was found to produce multicellular trichomes and it is postulated that the SIM product functions to repress mitosis in endocycles (Walker *et al.*, 2000). Importantly, *sim* trichome nuclei contained about one-third the DNA content of wild-type trichome nuclei yet, by contrast, epidermal pavement cell nuclei did not have a decreased amount of DNA relative to wild type, providing evidence for the existence of multiple pathways involved in the control of endoreduplication (Walker *et al.*, 2000). SIM was recently identified as a plant-specific protein with homology to the ICK/KRP family of cell cycle inhibitors (Churchman *et al.*, 2006). SIM contains a cyclin-binding domain and was shown to interact with D-type cyclins and CDKA;1 (Churchman *et al.*, 2006). In a different study, a D-type cyclin, *CYCD3;1*, was ectopically expressed in trichomes and produced multicellular trichomes like the *sim* mutant (Fig. 2) (Schnittger *et al.*, 2002a). *CYCD3;1* expression is normally not found in trichomes and therefore does not have a role in wild-type trichome development. Yet study of its ectopic expression revealed that it represses endoreduplication by promoting S-phase entry and inducing mitosis, a previously undefined role for D-type cyclins (Schnittger *et al.*, 2002a). Taken together, these data begin to provide the first evidence that SIM and D-type cyclins play an integral role in controlling the onset of endoreduplication in *Arabidopsis* trichomes. Similarly, ectopic B-cyclin *CYCB1;2* expression also transformed single-celled trichomes into multicellular hairs, promoting the G<sub>2</sub>-M phase transition (Schnittger *et al.*, 2002b). *CYCB1;2* may genetically interact with another component that represses its expression in trichomes, perhaps *SIM*; however, no evidence for this has been presented (Schnittger *et al.*, 2002b). Additionally, a cyclin-dependent kinase inhibitor, named ICK1/KRP1, was found to block entry into mitosis, promoting S-phase progression in a concentration-dependent manner (Schnittger *et al.*, 2003; Weinl *et al.*, 2005). ICK/KRP binds *CYCD3;1* in a yeast two-hybrid assay (Weinl *et al.*, 2005) and was able to correct the *sim* mutant phenotype (Weinl *et al.*, 2005). *cpr5* is another mutant in which the trichomes only undergo two rounds of endoreduplication, leading to decreased DNA levels (8C versus 32C) and less branching (Fig. 2) (Kirik *et al.*, 2001). Interestingly, pavement cell size is also affected in *cpr5*, suggesting that a pathway promoting endoreduplication functions in both pavement cells and trichomes (Kirik *et al.*, 2001). Much needs to be resolved regarding how endoreduplication controls cell size and morphology. It is still largely unclear how endoreduplication itself is regulated, and particularly how it is regulated in distinct cell types. There is ample



evidence for the existence of endoreduplication in *Arabidopsis* trichomes and pavement cells but minimal information available regarding the regulation of endoreduplication in root hairs (Larkin *et al.*, 2003; Sugimoto-Shirasu *et al.*, 2005). Undoubtedly, the utilization of trichomes, root hairs, and pavement cells in *Arabidopsis* to study endoreduplication will help to address some of these questions further.

### The cytoskeleton directs growth

The plant cytoskeleton (microtubules and microfilaments) and cell wall play an important role in cell shape formation in epidermal cells. During cell expansion and elongation, the cell wall is constantly being loosened and stiffened, conferring upon the cell the elasticity necessary to achieve growth. By directing the deposition of cell wall materials via secretion, the cytoskeleton influences where and in which direction cell growth takes place. While much remains to be elucidated about the mechanisms of cytoskeletal function in morphogenesis, recent findings have progressed further our understanding of how microtubules and actin filaments act to control cell polarity and expansion.

The targeted secretion of vesicles containing cell wall components has been associated with the tip-directed growth observed during root hair development. During this process, cell wall components are transported to and integrated in the tip area via the cytoskeleton, where high rates of both exocytosis and endocytosis are reported (Ueda *et al.*, 2004; Ovecka *et al.*, 2005; Smith and Oppenheimer, 2005). Actin has been shown to be an integral component of the mechanism involved in vesicle trafficking during rapid cell growth. A role for actin in the movement of endosomal compartments in the root tip was found recently (Voigt *et al.*, 2005). The root tip contains an enrichment of endosomal vesicles, and it was observed that the motility of these vesicles was dependent upon an intact actin cytoskeleton. Specifically, the endosomes were shown to move rapidly concomitant with the nucleation of actin filaments. This finding provides a new link between actin-driven polar tip growth and actin polymerization-propelled motility of endosomes (Voigt *et al.*, 2005). This makes sense when considering that an increase in exocytosis could result in a surplus of membrane material at the site of growth, and such excess membrane would need to be removed. Another possibility is that during cell wall remodelling, wall components need to be recycled and transferred to new sites of growth. It is likely that targeted secretion of vesicles is not only limited to tip-growth in root hairs, but also contributes to the more expansive growth of trichomes and pavement cells. The mechanism of how actin controls the directionality, motility, and specificity of vesicle trafficking during polar growth remains largely unclear, but some progress in understanding these dynamic processes has been made.

Additionally, a new role for the involvement of cortical microtubules in the control of cell growth and polarity is emerging. It has been proposed that endoplasmic microtubules function to establish a 'reinforcement patch' around the sites where the cortical F-actin cytoskeleton is weakened (Sieberer *et al.*, 2005; Mathur, 2006). These sites of cortical actin aggregation define regions for the recruitment of the endoplasmic microtubules (Sieberer *et al.*, 2005; Mathur, 2006). Furthermore, application of microtubule destabilizing drugs produces non-polarized, isotropically expanded cells, providing further support for this hypothesis (Mathur and Chua, 2000; Mathur *et al.*, 2002; Mathur, 2006).

### The ARP2/3 and Scar/WAVE complexes

As mentioned above, the actin cytoskeleton is a complex network that is involved in intercellular transport, organelle movement, and morphogenesis. These processes are dependent upon the polymerization of actin monomers into polymers, known as actin nucleation. In yeast and animals, actin nucleation and branching is mediated by the ARP2/3 complex (actin-related protein 2/3) and its activator complex called Scar/WAVE (suppressor of cyclic AMP receptor/Wiskott-Aldrich syndrome Verprolin-homologous protein). Components of the ARP2/3 complex and Scar/WAVE have been identified in higher plants and numerous reviews have been dedicated to the subject (Deeks and Hussey, 2003, 2005; Bannigan and Baskin, 2005; Mathur, 2005; Smith and Oppenheimer, 2005; Szymanski, 2005; Xu and Scheres, 2005; Mathur, 2006); therefore, they will be mentioned only briefly here, despite their importance in morphogenesis of epidermal cells.

The ARP2/3 complex consists of seven subunits: ARP2, ARP3, and ARPC1–C5). It binds to the sides of actin filaments and nucleates new filaments (Szymanski, 2005). An ARP2/3 complex has not been biochemically isolated from *Arabidopsis*, but homologues of each subunit are encoded in the *Arabidopsis* genome (Li *et al.*, 2003). Four of the seven homologues are encoded by the 'distorted' group of genes: *WRM* (ARP2), *DIS1* (ARP3), *DIS2* (ARPC2), and *CRK* (ARPC5) (Hulskamp *et al.*, 1994; Le *et al.*, 2003; Li *et al.*, 2003; Mathur *et al.*, 2003a, b; Schwab *et al.*, 2003; El-Din El-Assal *et al.*, 2004; Saedler *et al.*, 2004a). The distorted genes are a group of eight genes that were identified due to their F-actin organizational phenotypes, manifested as 'distorted' trichomes (Hulskamp *et al.*, 1994; Mathur *et al.*, 1999; Szymanski *et al.*, 1999; Schwab *et al.*, 2003). The remaining ARP2/3 complex genes, *ARPC1*, *ARPC3*, and *ARPC4*, are encoded and expressed in *Arabidopsis*; however, as far as is known, mutant alleles displaying a phenotype have not been obtained (Li *et al.*, 2003).

The Scar/WAVE complex consists of five subunits: ABI1, NAP1, PIR121, HSCP300, and the SCAR/WAVE protein itself. Plant homologues have also been identified for Scar/WAVE: BRK1 (HSCP300) (Frank and Smith, 2002; Djakovic *et al.*, 2006), NAP1 (GRL/NAPP) (Brembu *et al.*, 2004; Deeks *et al.*, 2004; El-Assal Sel *et al.*, 2004; Li *et al.*, 2004; Zimmermann *et al.*, 2004), SRA1 (KLK/PIRP/PIR121) (Basu *et al.*, 2004; Brembu *et al.*, 2004; Li *et al.*, 2004; Saedler *et al.*, 2004b), ABI1 (ABIL1) (Basu *et al.*, 2005), and SCAR/WAVE (DIS3/SCAR2/ITB1) (Basu *et al.*, 2005; Zhang *et al.*, 2005a, b). Two of the Scar/WAVE subunits, NAP1 and SRA1, belong to the distorted group genes. Like the ARP2/3 complex, the Scar/WAVE complex has not been biochemically isolated from plants. However, recent evidence showing that DIS3/SCAR2 was found to interact with ABI1 and NAPP/NAP1 provides evidence for its existence (Basu *et al.*, 2005). Furthermore, four new loci controlling trichome morphogenesis have been found recently; these have been named the *IRREGULAR TRICHOME BRANCH (ITB)* loci (Zhang *et al.*, 2005b). The *itb* mutants display branch position defects and have altered branch lengths (Fig. 2) (Zhang *et al.*, 2005b). *ITB1* was found to encode the Scar/WAVE subunit (Zhang *et al.*, 2005a) (see below); however, *ITB2*, 3, and 4 remain to be characterized.

In yeast and animals, the Scar/WAVE and ARP2/3 complexes are required for cell survival and contribute to the protrusion-mediated motility of cells and the subcellular motility of organelles and microbes (Mathur, 2005). In plants, the putative complexes might be involved in nucleation of actin during cell expansion, since actin dynamics are required for the intracellular trafficking of vesicles and subcellular components that is necessary to achieve cell growth (Mathur, 2005). However, as mutants are still viable, these complexes may not play an actin organizational role in all cell types (Szymanski, 2005).

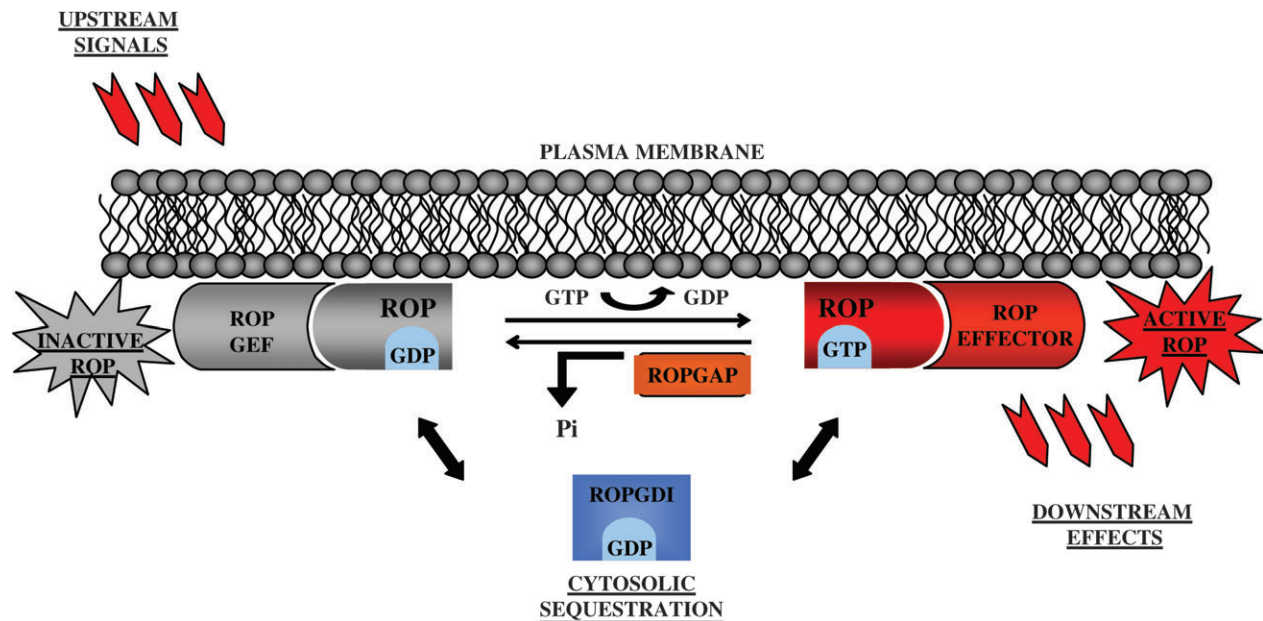
Like trichome and pavement cell expansion, growth of root hairs depends on actin polymerization (Hepler *et al.*, 2001). All the Scar/WAVE and ARP2/3 mutants contain highly distorted trichomes (Fig. 2) and aberrant pavement cells, but there are conflicting reports on whether or not these mutations affect the morphology of root hairs. Some groups report that root hair growth is not affected in Scar/WAVE-ARP2/3 mutants (Le *et al.*, 2003; Brembu *et al.*, 2004; El-Din El-Assal *et al.*, 2004; Djakovic *et al.*, 2006), while other groups found mild phenotypes (Li *et al.*, 2003; Mathur *et al.*, 2003a, b). Undoubtedly, the next necessary step will be to isolate the Scar/WAVE and ARP2/3 complexes from different epidermal cell tissues biochemically. This will answer whether it is universally utilized for actin nucleation in trichomes, pavement cells, and root hairs. Likewise, further studies on the two remaining uncharacterized distorted mutants, might yield new insights about the Scar/WAVE-ARP2/3 complexes in *Arabidopsis* and their contribution to epidermal cell morphogenesis.

## Small GTPase signalling cascades

RHO GTPases, one class of small GTPases, function as molecular switches between extracellular stimuli perception and signal transduction cascades. They are implicated in a variety of intracellular processes such as regulation of the actin cytoskeleton, gene expression, cell polarity, and the cell cycle (Jaffe and Hall, 2005). RHO GTPases can cycle between active GTP-bound and inactive GDP-bound states. The active and inactive status of RHO GTPases depends on numerous regulators. RHO GTPase signalling cascades are well characterized in animals and yeast, yet there has been little molecular evidence for their involvement in plant cell morphogenesis until recently. A specific subfamily of the RHO GTPases, called ROPs (for Rho of plants), is found in *Arabidopsis* and is composed of 11 members (Li *et al.*, 2001). Predicted regulators of ROPs include the guanine nucleotide exchange factors (ROPGEFs), GTPase-activating proteins (ROPGAPs), guanine nucleotide dissociation inhibitors (ROPGDIs), and ROP effector proteins (Gu *et al.*, 2004; Molendijk *et al.*, 2004; Xu and Scheres, 2005; Nibau *et al.*, 2006; Uhrig and Hulskamp, 2006) (see Fig. 3 for details). It is becoming clearer that ROPs and their regulators function as universal signalling components to modulate morphogenesis in epidermal cells.

### ROPs and their regulators

One of the best examples of ROP function in epidermal cell morphogenesis comes from studies on *ROP2*. *ROP2* is localized to root hair tips, and its overexpression results in depolarized, multi-tipped root hairs, suggesting its involvement in the establishment of sites for polarized growth (Jones *et al.*, 2002). Additionally, in dominant negative and constitutively active *ROP2* (*ROP2<sup>DN</sup>* and *ROP2<sup>CA</sup>*, respectively) expressing plants, many different cell types are distorted in comparison with wild type, including trichomes (Fu *et al.*, 2002). In *ROP2<sup>DN</sup>*, the formation of diffuse cortical fine F-actin is disrupted at regions of polarized growth, thus suggesting that *ROP2* controls cell expansion through the spatial regulation of cortical F-actin (Fu *et al.*, 2002). More recently, a complex *ROP2*- and *ROP4*-mediated signalling network in pavement cell lobe formation was elucidated (Fu *et al.*, 2005). In a tour de force, Fu *et al.* (2005) have shown that active GTP-bound *ROP2/4* interacts with *RIC1* and *RIC4*, two ROP effector proteins. This interaction results in two antagonistic pathways that have opposing effects on cell expansion (Fu *et al.*, 2005). *RIC1* was found to promote the organization of microtubule arrays that span neck-to-neck regions in pavement cells, thereby inhibiting lateral expansion in these regions. Concurrently, activated *ROP2/4* interacts with *RIC4* at expanding lobes, promoting the formation of fine F-actin while repressing *RIC1* at these same sites so that growth-restricting microtubules do not



**Fig. 3.** ROP regulation. Based on animal models, a hypothetical model for the regulation of ROP activity in plants is shown. ROP cycles between an inactive, primarily cytosolic, GDP-bound form and an active, membrane-associated GTP-bound form. ROPGEF stimulates the exchange of GDP for GTP, promoting the activation of ROP. ROPGAP stimulates the hydrolysis of the activated ROP, leading to its inactivation. ROPGDI functions in three ways. It can (i) inhibit the dissociation of GDP from ROP, maintaining the ROP in its inactive form, preventing ROP activation by ROPGEF, (ii) interact with the GTP-bound form of ROP to inhibit GTP hydrolysis, blocking GAP activity and interaction with effector targets, and (iii) modulate the cycling of ROP from the cytosol and the membrane.

form. It is not known how RIC1 is activated, but when activated, it not only promotes the formation of microtubules in the growth-restricted neck regions, but it also represses the ROP2/4–RIC4 pathway. In future studies, it will be interesting to investigate if the effect modulated by the ROP2/4–RIC4 pathway is mediated by the Scar/WAVE and/or ARP2/3 complex and to identify factor(s) that activate ROP2/4.

A possible activator of ROP2/4 might be the *SPK1* gene. *spk1* displays both trichome and pavement cell phenotypes similar to the ROP phenotypes (Qiu *et al.*, 2002; Frank *et al.*, 2003). Based on yeast two-hybrid data, it has been proposed that SPK1 is a ROP activator, yet it remains to be shown that this occurs *in vivo* (Qiu *et al.*, 2002; Uhrig *et al.*, 2007). Another possibility is that a ROP2/4 activator will be discovered amongst the recently identified 14-member family of ROPGEFs (Berken *et al.*, 2005; Gu *et al.*, 2006). ROPGEF1, was shown to activate ROP1 during pollen tube growth; therefore it is tempting to speculate that one or more ROPGEFs activate ROP2 in pavement cells and root hairs (Gu *et al.*, 2006).

Another possible regulator of ROP2/4 was found recently, a ROPGDI named *SCN1* (Parker *et al.*, 2000; Carol *et al.*, 2005). RhoGDIs normally bind Rho proteins, causing them to be sequestered away from the membrane and localized in the cytosol (Vernoud *et al.*, 2003). Upon release of binding, Rhos cycle back to the membrane where they are activated by RhoGEFs (Fig. 3). *SCN1* was shown to

regulate growth in root hair cells spatially by restricting the site of growth to a single point on the trichoblast (Carol *et al.*, 2005). It was found that *SCN1* determines where NADPH oxidase-derived reactive oxygen species (ROS) are produced (Carol *et al.*, 2005). RHD2 is an NADPH oxidase that has been shown to produce a tip-focused ROS gradient (Foreman *et al.*, 2003). The tip-focused ROS gradient is lost in *scn1* mutants, causing the root hair cells to form multiple bulges and tips along the length of the trichoblast cell (Carol *et al.*, 2005). Interestingly, ROP2 is mislocalized in *scn1* mutants, suggesting that *SCN1* regulates the spatial localization of both RHD2 and ROP2. However, for both cases, it remains to be determined if this regulation is direct or indirect.

Furthermore, studies on the planar polarization of root hairs in *Arabidopsis* revealed more upstream regulators of ROP2. Planar polarization, different from polarized tip growth, is the co-ordinated polarization of cells within a single layer of tissue, as can be found with root hair cells in the *Arabidopsis* epidermis (Sinnott and Bloch, 1939; Masucci and Schiefelbein, 1994; Grebe, 2004; Fischer *et al.*, 2007). They display planar polarity because all hairs are localized close to the basal end of the trichoblast (Masucci and Schiefelbein, 1994; Grebe, 2004; Fischer *et al.*, 2007). This polarization of the root hair initiation site coincides with a root-tip high auxin concentration gradient (Sabatini *et al.*, 1999; Grebe *et al.*, 2002) and polarized localization of ROPs (Fischer *et al.*,



2007). This acquisition of polarity within the trichoblast leading to basal polar root hair growth precedes tip growth. It was recently shown that the *AUX1*, *EIN2*, *ETO2*, *GNOM*, and *SUR2* genes act upstream of polar root hair positioning and polar ROP GTPase recruitment to the hair initiation site (Fischer *et al.*, 2007), revealing that their co-ordinated action characterizes an early step in root hair cell growth and differentiation.

In yeast and animals, Rho GTPases activate a plethora of distinct target effector proteins, which in turn activate numerous cellular responses. A similar scenario may exist for plant ROPs and effector targets. Providing evidence for such a pathway in plants, a novel ROP effector was identified that links ROPs to vesicle trafficking and polarity. ICR1 (interactor of constitutive active ROPs 1) defines a novel class of ROP effectors in plants (Lavy *et al.*, 2007). It was found to interact with some GTP-bound ROPs (ROP6, ROP8) and, when ectopically expressed, plants displayed deformed pavement and root hair cells (Lavy *et al.*, 2007). Furthermore, ICR1 can bind AtSEC3 and ROP simultaneously. In yeast, SEC3 is a subunit of the exocyst complex, which functions to tether vesicles to membranes during exocytosis (TerBush *et al.*, 1996). Since ICR1 can bind ROP and AtSEC3 simultaneously, it was proposed that it functions as a membrane scaffold protein, mediating the interaction of ROPs with other regulatory proteins. In yeast, the exocyst complex interacts with the ARP2/3 complex, linking secretion with actin organization (Lavy *et al.*, 2007). It is possible that ICR1, AtSEC3, and ROP form a similar complex in *Arabidopsis*, interacting with the ARP2/3 complex. Taken together with the data on the ROP2/4–RIC1/4 pathways, a clearer picture is starting to emerge of how ROPs and their effectors possibly signal to downstream cellular components such as the actin organization and vesicle trafficking machinery. Importantly, Uhrig *et al.* (2007) recently undertook the large task of testing all possible protein–protein interaction combinations with ARP2/3, Scar/WAVE, and ROP proteins from *Arabidopsis* (Uhrig *et al.*, 2007). Utilizing a combination of both *in vitro* and *in vivo* assays, their data reveal novel interactions within the ARP2/3 and Scar/WAVE complexes, as well as new interactions with ROPs (Uhrig *et al.*, 2007). Their data also suggest that SPIKE1 could actually be one of the components of the Scar/WAVE complex, acting as a direct effector of ROPs (Uhrig *et al.*, 2007). A summary of their protein–protein interaction data can be found in Table 1.

Another gene that could be involved in linking vesicle trafficking and polarity with ROPs is *TIP1* (Schiefelbein *et al.*, 1993; Hemsley *et al.*, 2005). Root hairs of the *tip1* mutant are shorter and often branched, with up to four hairs emerging from one initiation site (Hemsley *et al.*, 2005). *TIP1* encodes an *S*-acyl transferase (also known as palmitoyl transferase). Protein palmitoylation is a revers-

ible modification that can affect protein association with membranes, signal transduction, and vesicle trafficking within cells (Hemsley *et al.*, 2005). The loss of polarity seen in *tip1* is reminiscent of the ROP2 overexpressor phenotype, which suggests that TIP1 acetylates ROP2, targeting it to the membrane, although this remains highly speculative (Jones *et al.*, 2002). It will be interesting to see if further characterization can reveal that TIP1 is involved in targeting proteins to the membrane or directing vesicle traffic during the growth of root hairs and other epidermal cells.

### Rab GTPases

In animals and yeast, Rab GTPases are involved in several different steps of membrane trafficking. They are responsible for the docking of vesicles on the cytosolic face of many intracellular membranes (Hepler *et al.*, 2001) and anchoring actin- and tubulin-delivered vesicles via their effector proteins before their subsequent vesicle-membrane fusion (Hepler *et al.*, 2001; Stenmark and Olkkonen, 2001; Molendijk *et al.*, 2004). A few recent publications highlight the importance of Rab GTPases in the targeted secretion of cell-wall components during epidermal morphogenesis (Rutherford and Moore, 2002; Vernoud *et al.*, 2003).

In developing root hair cells, a novel, undefined membrane compartment has been identified. It was found that AtRabA4b GTPase was associated with both Golgi membrane markers and these novel compartments (Preuss *et al.*, 2004). These compartments did not contain Golgi or trans-Golgi network markers and were located at the tips of expanding root hairs. Their polarized distribution was altered by treatment with actin-disrupting drugs, indicating that their localization to root hair tips was dependent on F-actin. Treatment with a  $\text{Ca}^{2+}$  ionophore, which disrupts the tip-focused  $\text{Ca}^{2+}$  gradient, caused a rapid dispersal of the markers labelling the novel compartments (Preuss *et al.*, 2006). Moreover, the membrane compartments did not localize to other known regions of membrane expansion, suggesting that they have a role in secretion of cell wall materials. Taken together, the localization of AtRabA4b GTPase at the Golgi and to novel tip-localized compartments suggests that it plays a role in cell wall cargo sorting and trafficking between the two, and that this trafficking relies on the actin cytoskeleton (Preuss *et al.*, 2004). An AtRabA4b effector protein, phosphatidylinositol 4-OH kinase (PI-4K $\beta$ 1) specifically interacts with GTP-bound AtRabA4b and colocalizes with the novel membrane compartments previously identified (Preuss *et al.*, 2004, 2006). Additionally, AtRabA4b has no obvious phenotype (Preuss *et al.*, 2004), although the PI-4K $\beta$ 1/PI-4K $\beta$ 2 double mutant displayed aberrant root hairs. PI-4K $\beta$ 1 was also found to interact with a calcineurin B-like protein (AtCBL1), a  $\text{Ca}^{2+}$  sensor. The localization of RabA4b-labelled



**Table 1.** *ARP2/3, Scar/WAVE, and ROP protein–protein interactions*

This is a summary of protein–protein interactions taken from Uhrig *et al.* (2007) and reproduced by kind permission of the Company of Biologists. Proteins implicated in the putative *Arabidopsis* ARP2/3 and Scar/WAVE complexes are listed in the far left column. The three right-most columns indicate which proteins were found to interact in yeast two-hybrid (Y2H) and/or bimolecular fluorescence complementation (BiFC) experiments. Italicized type indicates interaction only from Y2H experiments (*in vitro* only). Bold type indicates interaction for Y2H and co-localization in BiFC experiments (*in vitro* and *in vivo*). (+), Presence of a protein–protein interaction; (–), absence of protein–protein interaction in both Y2H and BiFC.

	Interactions with ARP2/3 complex genes	Interactions with Scar/WAVE complex genes and homologues	Interactions with ROPs*
<b>ARP2/3 genes</b>			
<b>ARP2</b>	(+) <i>ARP3</i> (–) <i>ARP2, ARPC2, ARPC3, ARPC4</i>	<i>ABI1</i> <i>BRICK1, SCAR1</i>	nd <i>ROP8<sup>CA</sup></i>
<b>ARP3</b>	(+) <i>ARP2</i> (–) <i>ARP3</i>	<b><i>BRICK1, ABI1, ABI2, ABI4, SCAR3</i></b> <i>SCAR2</i>	nd nd
<b>ARPC1</b>	(+) <i>ARPC4</i> (–) nd	nd nd	nd nd
<b>ARPC2</b>	(+) <b><i>ARPC4, SCAR3</i></b> (–) <i>ARP2, ARPC3</i>	nd nd	nd <i>ROP7, ROP7<sup>CA</sup>, ROP8</i>
<b>ARPC3</b>	(+) <b><i>ARPC4</i></b> (–) <i>ARP2, ARPC2, ARPC3</i>	<b><i>BRICK1, SCAR1, SCAR2, ABI1, ABI2, SCAR3</i></b> <i>ABI4</i>	nd nd
<b>ARPC4</b>	(+) <b><i>ARPC2, ARPC3, ARPC1, ARPC5</i></b> (–) <i>ARP2</i>	nd <i>SCAR4</i>	nd nd
<b>ARPC5</b>	(+) <i>ARPC4</i> (–) nd	nd nd	nd nd
<b>Scar/WAVE genes</b>			
<b>NAP125</b>	(+) nd (–) nd	<i>NAP125, KLK, ABI1, ABI2</i> nd	nd nd
<b>KLK</b>	(+) nd (–) nd	<i>NAP125</i> nd	nd nd
<b>ABI1</b>	(+) <i>ARP2, ARP3, ARPC3</i> (–) <i>ABI1</i>	<b><i>SCAR2, SPIKE1, NAP125, BRICK1, SCAR1, SCAR3, SCAR-like</i></b> nd	nd nd
<b>ABI2</b>	(+) <i>ARP3, ARPC3</i> (–) nd	<i>NAP125, BRICK1, SCAR1, SCAR2, SCAR3, SCAR-like, SPIKE1</i> nd	nd nd
<b>ABI3</b>	(+) nd (–) nd	<i>BRICK1, SCAR3, SPIKE1</i> nd	nd nd
<b>ABI4</b>	(+) <i>ARP3</i> (–) <i>ARPC3</i>	<b><i>BRICK1, SCAR2, SCAR3, SPIKE1</i></b> nd	nd nd
<b>BRICK1</b>	(+) <b><i>ARP3, ARPC3,</i></b> (–) <i>ARP2</i>	<b><i>BRICK1, ABI4, SCAR2, SCAR3, ABI1, ABI2, ABI3, SCAR1</i></b> nd	<i>ROP2, ROP7, ROP7<sup>CA</sup>, ROP8 CA</i> <i>ROP5, ROP8, ROP11<sup>CA</sup></i>
<b>SCAR1</b>	(+) <b><i>ARPC3</i></b> (–) <i>ARP2</i>	<b><i>SCAR2, SCAR3, ABI1, ABI2, BRICK1, SPIKE1</i></b> nd	<i>ROP7, ROP7<sup>CA</sup>, ROP8<sup>CA</sup></i> <b><i>ROP7, ROP7<sup>CA</sup>, ROP8, ROP8<sup>CA</sup>, ROP5, ROP11 CA</i></b> <i>ROP2<sup>CA</sup>, ROP7<sup>DN</sup></i>
<b>SCAR2</b>	(+) <b><i>ARPC3</i></b> (–) <i>ARP3</i>	<b><i>ABI1, ABI4, BRICK1, SPIKE1, ABI2,</i></b> nd	<i>ROP8, ROP8<sup>CA</sup></i> <i>ROP7, ROP7<sup>CA</sup></i> <i>ROP5, ROP11<sup>CA</sup></i> <i>ROP2, ROP2<sup>CA</sup>, ROP7<sup>CA</sup></i> <i>ROP8</i>
<b>SCAR3</b>	(+) <b><i>ARPC2, ARP3, ARPC3</i></b> (–) nd	<b><i>ABI4, BRICK1, ABI1, ABI2, ABI3, SPIKE1</i></b> nd	<i>ROP2, ROP2<sup>CA</sup>, ROP7<sup>CA</sup></i> nd
<b>SCAR4</b>	(+) nd (–) <i>ARPC4</i>	<i>SPIKE1</i> nd	<i>ROP2, ROP5, ROP8, ROP11<sup>CA</sup></i> <i>SCAR1, SCAR3, SCAR4</i>
<b>SCAR-like</b>	(+) nd (–) nd	<i>ABI1, ABI2</i> nd	<i>ROP2<sup>CA</sup>, ROP7, ROP7<sup>CA</sup></i>
<b>SPIKE1</b>	(+) nd (–) nd	<b><i>SPIKE1, ABI1, SCAR2, ABI2, ABI3, ABI4, SCAR1, SCAR3, SCAR4</i></b> nd	

\* CA, Constitutively active; DN, dominant negative.

membrane compartments to root hair tips could be dependent on the recruitment and Ca<sup>2+</sup> activation of PI-4Kβ1 to these compartments. Upon recruitment to this compartment, PI-4Kβ1 could increase the production of phosphatidylinositol 4'-monophosphates (PI-4P), a phosphoinositide molecule. These molecules can mark sub-

domains within a membrane, and, in the case of root hair tip growth, PI-4P might be involved in organizing post-Golgi secretory compartments to the tips of growing hairs. For the future, it will be interesting to see if any Rab activity is observable in pavement cell lobe growth or in the growth of trichome branches.

## Conclusions and perspectives

Cell morphogenesis is dynamic and complex, involving the concerted action of multiple cellular processes. The final shapes and size of plant cells are a crucial determinant of plant organ function. This idea certainly holds true for the epidermis of *Arabidopsis*, which contains a collection of different cells with extremely different shapes and functions. A slew of mutants have been identified with defects in epidermal cell morphogenesis for which a biological role has not been assigned (see Table S1 in the Supplementary data at JXB online). Further detailed characterization of these proteins will surely add to the growing body of knowledge forming on how cell shape is determined in the plant epidermis and in other plant cells. Genetic screens to identify further morphology mutants, especially pavement cell mutants (for there is a paucity of mutants for this particular cell type) will shed light on how cell shape and polarity is controlled in disparate cell types. A challenge for the future will be to determine which mechanisms are conserved amongst the different cell types and which remain specific to each.

Many important questions about epidermal cell morphogenesis remain to be addressed. For example, how is endoreduplication regulated and is it differentially regulated in different cell types? Why do some plant species exhibit more endoreduplication than others? Is endoreduplication linked to control of the cytoskeleton and vesicle trafficking? Endoreduplication occurs throughout the plant and is crucial for cell growth yet it is not as widely studied in plants as one would expect for such an important phenomenon. More investigations to identify mutants defective in endoreduplication are needed.

Furthermore, it will be interesting to see if the remaining uncharacterized distorted mutants *ali* and *spi* are part of the Scar/WAVE and/or ARP2/3 complexes or if they are involved in other actin-related processes. Moreover, the biochemical isolation of both the Scar/WAVE and ARP2/3 complexes needs to be accomplished as their isolation will surely provide critical information for elucidation of actin function in cell growth. Additionally, as the mechanistic details of how ROPs regulate cytoskeletal dynamics still remain unclear, more ROP downstream effectors will need to be identified and their functions characterized. Along with their identification, isolation of novel ROP activators should provide more information on how ROPs transmit extracellular signals to the cytoskeleton. Promoter studies to identify the different tissue specificities of the different ROPs might prove useful to determine if there are overlapping morphological mechanisms in different cells. Lastly, Rab GTPase involvement in the organization of post-Golgi secretory compartments needs to be experimentally verified. It is certain that in the coming years a multitude of groups will help to address

these fundamental questions regarding epidermal cell shape formation.

## Supplementary data

Supplementary data can be found at JXB online. It consists of **Table S1** which is a list of genes implicated in the morphogenesis of trichomes, root hairs, and pavement cells.

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## References

- Bannigan A, Baskin TI. 2005. Directional cell expansion – turning toward actin. *Current Opinion in Plant Biology* **8**, 619–624.
- Basu D, El-Assal Sel D, Le J, Mallery EL, Szymanski DB. 2004. Interchangeable functions of Arabidopsis PIROGI and the human WAVE complex subunit SRA1 during leaf epidermal development. *Development* **131**, 4345–4355.
- Basu D, Le J, El-Assal Sel D, Huang S, Zhang C, Mallery EL, Koliantz G, Staiger CJ, Szymanski DB. 2005. DISTORTED3/SCAR2 is a putative arabidopsis WAVE complex subunit that activates the Arp2/3 complex and is required for epidermal morphogenesis. *The Plant Cell* **17**, 502–524.
- Berken A, Thomas C, Wittinghofer A. 2005. A new family of RhoGEFs activates the Rop molecular switch in plants. *Nature* **436**, 1176–1180.
- Brembu T, Winge P, Seem M, Bones AM. 2004. NAPP and PIRP encode subunits of a putative wave regulatory protein complex involved in plant cell morphogenesis. *The Plant Cell* **16**, 2335–2349.
- Carol RJ, Takeda S, Linstead P, Durrant MC, Kakesova H, Derbyshire P, Drea S, Zarsky V, Dolan L. 2005. A RhoGDP dissociation inhibitor spatially regulates growth in root hair cells. *Nature* **438**, 1013–1016.
- Churchman ML, Brown ML, Kato N, *et al.* 2006. SIAMESE, a plant-specific cell cycle regulator, controls endoreplication onset in *Arabidopsis thaliana*. *The Plant Cell* **18**, 3145–3157.
- Deeks MJ, Hussey PJ. 2003. Arp2/3 and ‘the shape of things to come’. *Current Opinion in Plant Biology* **6**, 561–567.
- Deeks MJ, Hussey PJ. 2005. Arp2/3 and SCAR: plants move to the fore. *Nature Reviews Molecular Cell Biology* **6**, 954–964.
- Deeks MJ, Kaloriti D, Davies B, Malho R, Hussey PJ. 2004. Arabidopsis NAP1 is essential for Arp2/3-dependent trichome morphogenesis. *Current Biology* **14**, 1410–1414.
- Djakovic S, Dyachok J, Burke M, Frank MJ, Smith LG. 2006. BRICK1/HSPC300 functions with SCAR and the ARP2/3 complex to regulate epidermal cell shape in Arabidopsis. *Development* **133**, 1091–1100.
- El-Assal Sel D, Le J, Basu D, Mallery EL, Szymanski DB. 2004. Arabidopsis GNARLED encodes a NAP125 homolog that positively regulates ARP2/3. *Current Biology* **14**, 1405–1409.
- El-Din El-Assal S, Le J, Basu D, Mallery EL, Szymanski DB. 2004. DISTORTED2 encodes an ARPC2 subunit of the putative Arabidopsis ARP2/3 complex. *The Plant Journal* **38**, 526–538.

- El Refy A, Perazza D, Zekraoui L, Valay JG, Bechtold N, Brown S, Hulskamp M, Herzog M, Bonneville JM. 2003. The Arabidopsis KAKTUS gene encodes a HECT protein and controls the number of endoreduplication cycles. *Molecular Genetics and Genomics* **270**, 403–414.
- Fischer U, Ikeda Y, Grebe M. 2007. Planar polarity of root hair positioning in Arabidopsis. *Biochemical Society Transactions* **35**, 149–151.
- Folkers U, Berger J, Hulskamp M. 1997. Cell morphogenesis of trichomes in Arabidopsis: differential control of primary and secondary branching by branch initiation regulators and cell growth. *Development* **124**, 3779–3786.
- Foreman J, Demidchik V, Bothwell JH, et al. 2003. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature* **422**, 442–446.
- Frank MJ, Cartwright HN, Smith LG. 2003. Three Brick genes have distinct functions in a common pathway promoting polarized cell division and cell morphogenesis in the maize leaf epidermis. *Development* **130**, 753–762.
- Frank MJ, Smith LG. 2002. A small, novel protein highly conserved in plants and animals promotes the polarized growth and division of maize leaf epidermal cells. *Current Biology* **12**, 849–853.
- Fu Y, Gu Y, Zheng Z, Wasteney G, Yang Z. 2005. Arabidopsis interdigitating cell growth requires two antagonistic pathways with opposing action on cell morphogenesis. *Cell* **120**, 687–700.
- Fu Y, Li H, Yang Z. 2002. The ROP2 GTPase controls the formation of cortical fine F-actin and the early phase of directional cell expansion during Arabidopsis organogenesis. *The Plant Cell* **14**, 777–794.
- Grebe M. 2004. Ups and downs of tissue and planar polarity in plants. *Bioessays* **26**, 719–729.
- Grebe M, Friml J, Swarup R, Ljung K, Sandberg G, Terlou M, Palme K, Bennett MJ, Scheres B. 2002. Cell polarity signaling in Arabidopsis involves a BFA-sensitive auxin influx pathway. *Current Biology* **12**, 329–334.
- Grierson C, Schiefelbein J. 2002. Root hairs. In: Somerville CR, Meyerowitz EM, eds. *The Arabidopsis book*. Rockville, MD: American Society of Plant Biologists, 1–22.
- Gu Y, Li S, Lord EM, Yang Z. 2006. Members of a novel class of Arabidopsis Rho guanine nucleotide exchange factors control Rho GTPase-dependent polar growth. *The Plant Cell* **18**, 366–381.
- Gu Y, Wang Z, Yang Z. 2004. ROP/RAC GTPase: an old new master regulator for plant signaling. *Current Opinion in Plant Biology* **7**, 527–536.
- Hemsley PA, Kemp AC, Grierson CS. 2005. The TIP GROWTH DEFECTIVE1 S-acyl transferase regulates plant cell growth in Arabidopsis. *The Plant Cell* **17**, 2554–2563.
- Hepler PK, Vidali L, Cheung AY. 2001. Polarized cell growth in higher plants. *Annual Review of Cell and Developmental Biology* **17**, 159–187.
- Hulskamp M, Misra S, Jurgens G. 1994. Genetic dissection of trichome cell development in Arabidopsis. *Cell* **76**, 555–566.
- Jaffe AB, Hall A. 2005. Rho GTPases: biochemistry and biology. *Annual Review of Cell and Developmental Biology* **21**, 247–269.
- Jones MA, Shen JJ, Fu Y, Li H, Yang Z, Grierson CS. 2002. The Arabidopsis Rop2 GTPase is a positive regulator of both root hair initiation and tip growth. *The Plant Cell* **14**, 763–776.
- Kirik V, Bouyer D, Schobinger U, Bechtold N, Herzog M, Bonneville JM, Hulskamp M. 2001. CPR5 is involved in cell proliferation and cell death control and encodes a novel transmembrane protein. *Current Biology* **11**, 1891–1895.
- Larkin JC, Brown ML, Schiefelbein J. 2003. How do cells know what they want to be when they grow up? Lessons from epidermal patterning in Arabidopsis. *Annual Review of Plant Biology* **54**, 403–430.
- Lavy M, Bloch D, Hazak O, Gutman I, Poraty L, Sorek N, Sternberg H, Yalovsky S. 2007. A novel ROP/RAC effector links cell polarity, root-meristem maintenance, and vesicle trafficking. *Current Biology*.
- Le J, El-Assal Sel D, Basu D, Saad ME, Szymanski DB. 2003. Requirements for Arabidopsis ATARP2 and ATARP3 during epidermal development. *Current Biology* **13**, 1341–1347.
- Li H, Shen JJ, Zheng ZL, Lin Y, Yang Z. 2001. The Rop GTPase switch controls multiple developmental processes in Arabidopsis. *Plant Physiology* **126**, 670–684.
- Li S, Blanchoin L, Yang Z, Lord EM. 2003. The putative Arabidopsis arp2/3 complex controls leaf cell morphogenesis. *Plant Physiology* **132**, 2034–2044.
- Li Y, Sorefan K, Hemmann G, Bevan MW. 2004. Arabidopsis NAP and PIR regulate actin-based cell morphogenesis and multiple developmental processes. *Plant Physiology* **136**, 3616–3627.
- Masucci JD, Schiefelbein JW. 1994. The rhd6 mutation of *Arabidopsis thaliana* alters root-hair initiation through an auxin- and ethylene-associated process. *Plant Physiology* **106**, 1335–1346.
- Mathur J. 2004. Cell shape development in plants. *Trends in Plant Science* **9**, 583–590.
- Mathur J. 2005. The ARP2/3 complex: giving plant cells a leading edge. *Bioessays* **27**, 377–387.
- Mathur J. 2006. Local interactions shape plant cells. *Current Opinion in Cell Biology* **18**, 40–46.
- Mathur J, Chua NH. 2000. Microtubule stabilization leads to growth reorientation in Arabidopsis trichomes. *The Plant Cell* **12**, 465–477.
- Mathur J, Mathur N, Hulskamp M. 2002. Simultaneous visualization of peroxisomes and cytoskeletal elements reveals actin and not microtubule-based peroxisome motility in plants. *Plant Physiology* **128**, 1031–1045.
- Mathur J, Mathur N, Kernebeck B, Hulskamp M. 2003a. Mutations in actin-related proteins 2 and 3 affect cell shape development in Arabidopsis. *The Plant Cell* **15**, 1632–1645.
- Mathur J, Mathur N, Kirik V, Kernebeck B, Srinivas BP, Hulskamp M. 2003b. Arabidopsis CROOKED encodes for the smallest subunit of the ARP2/3 complex and controls cell shape by region specific fine F-actin formation. *Development* **130**, 3137–3146.
- Mathur J, Spielhofer P, Kost B, Chua N. 1999. The actin cytoskeleton is required to elaborate and maintain spatial patterning during trichome cell morphogenesis in *Arabidopsis thaliana*. *Development* **126**, 5559–5568.
- Melaragno JE, Mehrotra B, Coleman AW. 1993. Relationship between endopolyplidy and cell size in epidermal tissue of Arabidopsis. *The Plant Cell* **5**, 1661–1668.
- Molendijk AJ, Ruperti B, Palme K. 2004. Small GTPases in vesicle trafficking. *Current Opinion in Plant Biology* **7**, 694–700.
- Nibau C, Wu HM, Cheung AY. 2006. RAC/ROP GTPases: ‘hubs’ for signal integration and diversification in plants. *Trends in Plant Science* **11**, 309–315.
- Ovecka M, Lang I, Baluska F, Ismail A, Illes P, Lichtscheidl IK. 2005. Endocytosis and vesicle trafficking during tip growth of root hairs. *Protoplasma* **226**, 39–54.
- Parker JS, Cavell AC, Dolan L, Roberts K, Grierson CS. 2000. Genetic interactions during root hair morphogenesis in Arabidopsis. *The Plant Cell* **12**, 1961–1974.
- Passardi F, Dobias J, Valerio L, Guimil S, Penel C, Dunand C. 2007. Morphological and physiological traits of three major *Arabidopsis thaliana* accessions. *Journal of Plant Physiology* **164**, 980–992.



- Perazza D, Herzog M, Hulskamp M, Brown S, Dorne AM, Bonneville JM. 1999. Trichome cell growth in *Arabidopsis thaliana* can be derepressed by mutations in at least five genes. *Genetics* **152**, 461–476.
- Preuss ML, Schmitz AJ, Thole JM, Bonner HK, Otegui MS, Nielsen E. 2006. A role for the RabA4b effector protein PI-4K $\beta$ 1 in polarized expansion of root hair cells in *Arabidopsis thaliana*. *Journal of Cell Biology* **172**, 991–998.
- Preuss ML, Serna J, Falbel TG, Bednarek SY, Nielsen E. 2004. The *Arabidopsis* Rab GTPase RabA4b localizes to the tips of growing root hair cells. *The Plant Cell* **16**, 1589–1603.
- Qiu JL, Jilk R, Marks MD, Szymanski DB. 2002. The *Arabidopsis* SPIKE1 gene is required for normal cell shape control and tissue development. *The Plant Cell* **14**, 101–118.
- Ramsay NA, Glover BJ. 2005. MYB-bHLH-WD40 protein complex and the evolution of cellular diversity. *Trends in Plant Science* **10**, 63–70.
- Rutherford S, Moore I. 2002. The *Arabidopsis* Rab GTPase family: another enigma variation. *Current Opinion in Plant Biology* **5**, 518–528.
- Sabatini S, Beis D, Wolkenfelt H, *et al.* 1999. An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. *Cell* **99**, 463–472.
- Saedler R, Mathur N, Srinivas BP, Kernebeck B, Hulskamp M, Mathur J. 2004a. Actin control over microtubules suggested by DISTORTED2 encoding the *Arabidopsis* ARP2C subunit homolog. *Plant Cell Physiology* **45**, 813–822.
- Saedler R, Zimmermann I, Mutondo M, Hulskamp M. 2004b. The *Arabidopsis* KLUNKER gene controls cell shape changes and encodes the AtSRA1 homolog. *Plant Molecular Biology* **56**, 775–782.
- Schellmann S, Hulskamp M. 2005. Epidermal differentiation: trichomes in *Arabidopsis* as a model system. *The International Journal of Developmental Biology* **49**, 579–584.
- Schiefelbein J, Galway M, Masucci J, Ford S. 1993. Pollen tube and root-hair tip growth is disrupted in a mutant of *Arabidopsis thaliana*. *Plant Physiology* **103**, 979–985.
- Schnittger A, Schobinger U, Bouyer D, Weinl C, Stierhof YD, Hulskamp M. 2002a. Ectopic D-type cyclin expression induces not only DNA replication but also cell division in *Arabidopsis* trichomes. *Proceedings of the National Academy of Sciences, USA* **99**, 6410–6415.
- Schnittger A, Schobinger U, Stierhof YD, Hulskamp M. 2002b. Ectopic B-type cyclin expression induces mitotic cycles in endoreduplicating *Arabidopsis* trichomes. *Current Biology* **12**, 415–420.
- Schnittger A, Weinl C, Bouyer D, Schobinger U, Hulskamp M. 2003. Misexpression of the cyclin-dependent kinase inhibitor ICK1/KRP1 in single-celled *Arabidopsis* trichomes reduces endoreduplication and cell size and induces cell death. *The Plant Cell* **15**, 303–315.
- Schwab B, Mathur J, Saedler R, Schwarz H, Frey B, Scheidegger C, Hulskamp M. 2003. Regulation of cell expansion by the DISTORTED genes in *Arabidopsis thaliana*: actin controls the spatial organization of microtubules. *Molecular Genetics and Genomics* **269**, 350–360.
- Sieberer BJ, Ketelaar T, Esseling JJ, Emons AM. 2005. Microtubules guide root hair tip growth. *New Phytologist* **167**, 711–719.
- Sinnott EW, Bloch R. 1939. Cell polarity and the differentiation of root hairs. *Proceedings of the National Academy of Sciences, USA* **25**, 248–252.
- Smith LG, Oppenheimer DG. 2005. Spatial control of cell expansion by the plant cytoskeleton. *Annual Review of Cell and Developmental Biology* **21**, 271–295.
- Stenmark H, Olkkonen VM. 2001. The Rab GTPase family. *Genome Biology* **2**, Reviews3007.1–3007.7.
- Sugimoto-Shirasu K, Roberts GR, Stacey NJ, McCann MC, Maxwell A, Roberts K. 2005. RHL1 is an essential component of the plant DNA topoisomerase VI complex and is required for ploidy-dependent cell growth. *Proceedings of the National Academy of Sciences, USA* **102**, 18736–18741.
- Sugimoto-Shirasu K, Roberts K. 2003. ‘Big it up’: endoreduplication and cell-size control in plants. *Current Opinion in Plant Biology* **6**, 544–553.
- Szymanski DB. 2005. Breaking the WAVE complex: the point of *Arabidopsis* trichomes. *Current Opinion in Plant Biology* **8**, 103–112.
- Szymanski DB, Marks MD, Wick SM. 1999. Organized F-actin is essential for normal trichome morphogenesis in *Arabidopsis*. *The Plant Cell* **11**, 2331–2347.
- TerBush DR, Maurice T, Roth D, Novick P. 1996. The Exocyst is a multiprotein complex required for exocytosis in *Saccharomyces cerevisiae*. *EMBO Journal* **15**, 6483–6494.
- Ueda T, Uemura T, Sato MH, Nakano A. 2004. Functional differentiation of endosomes in *Arabidopsis* cells. *The Plant Journal* **40**, 783–789.
- Uhrig JF, Hulskamp M. 2006. Plant GTPases: regulation of morphogenesis by ROPs and ROS. *Current Biology* **16**, R211–R213.
- Uhrig JF, Mutondo M, Zimmermann I, Deeks MJ, Machesky LM, Thomas P, Uhrig S, Rambke C, Hussey PJ, Hulskamp M. 2007. The role of *Arabidopsis* SCAR genes in ARP2-ARP3-dependent cell morphogenesis. *Development* **134**, 967–977.
- Vernoud V, Horton AC, Yang Z, Nielsen E. 2003. Analysis of the small GTPase gene superfamily of *Arabidopsis*. *Plant Physiology* **131**, 1191–1208.
- Voigt B, Timmers AC, Samaj J, *et al.* 2005. Actin-based motility of endosomes is linked to the polar tip growth of root hairs. *European Journal of Cell Biology* **84**, 609–621.
- Walker JD, Oppenheimer DG, Concienne J, Larkin JC. 2000. SIAMESE, a gene controlling the endoreduplication cell cycle in *Arabidopsis thaliana* trichomes. *Development* **127**, 3931–3940.
- Weinl C, Marquardt S, Kuijt SJ, Nowack MK, Jakoby MJ, Hulskamp M, Schnittger A. 2005. Novel functions of plant cyclin-dependent kinase inhibitors, ICK1/KRP1, can act non-cell-autonomously and inhibit entry into mitosis. *The Plant Cell* **17**, 1704–1722.
- Xu J, Scheres B. 2005. Cell polarity: ROPing the ends together. *Current Opinion in Plant Biology* **8**, 613–618.
- Zhang X, Dyachok J, Krishnakumar S, Smith LG, Oppenheimer DG. 2005a. IRREGULAR TRICHOME BRANCH1 in *Arabidopsis* encodes a plant homolog of the actin-related protein2/3 complex activator Scar/WAVE that regulates actin and microtubule organization. *The Plant Cell* **17**, 2314–2326.
- Zhang X, Grey PH, Krishnakumar S, Oppenheimer DG. 2005b. The IRREGULAR TRICHOME BRANCH loci regulate trichome elongation in *Arabidopsis*. *Plant Cell Physiology* **46**, 1549–1560.
- Zimmermann I, Saedler R, Mutondo M, Hulskamp M. 2004. The *Arabidopsis* GNARLED gene encodes the NAP125 homolog and controls several actin-based cell shape changes. *Molecular Genetics and Genomics* **272**, 290–296.